



RESEARCH ARTICLE

DEVELOPMENT OF MICROSPHERES CONTAINING DICLOFENAC DIETHYLAMINE AS SUSTAINED RELEASE TOPICAL FORMULATION

Ganesh Dinkarrao Basarkar*, Gunwant Narendra Shirsath and Sanjay Bhivasan Patil

Department of Pharmaceutics, SNJB's Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad, Nashik-423 101, Maharashtra, India

*E-mail: basarkarg@yahoo.com

Tel.: +91 9765942836.

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The aim of present work was to formulate diclofenac diethylamine microspheres using a natural wax, to be applied topically on the skin for the purpose of sustaining its release to avoid the side effects resulting from the oral administration of the drug and also to reduce the dosing frequency. Wax collected was purified using reported method and evaluated for physicochemical parameters. Drug excipients compatibility was performed using IR and DSC study. Following preliminary evaluations on process conditions for preparation of microspheres by cooling induced solidification technique, a 3² full factorial design was employed to investigate the influence of the formulation variables like concentration of wax and concentration of Tween 80 on the particle size, entrapment efficiency and drug release. Developed formulation followed Higuchi model for drug release from microspheres. Further, these microspheres were dispersed in carbopol 934 gel (1% w/w). The gel was evaluated for appearance, homogeneity, pH, spreadability, viscosity, drug content uniformity and *in vitro* drug diffusion study. Korsmeyer-Peppas equation was followed for *in vitro* drug diffusion from gel containing microspheres. Diffusion coefficient of Korsmeyer-Peppas equation indicated that the non-Fickian mechanism was basically involved in the drug release from gel containing microspheres.

Key words: Diclofenac diethylamine, Sustained release microspheres, Cooling induced solidification technique, Rice bran wax.

INTRODUCTION

In recent years, it has become more and more evident that the development of new drugs alone is not sufficient to ensure progress in drug therapy. A promising strategy involves the development of suitable drug carrier systems. Lipid particles based on triglycerides, waxes or fatty acids as matrix lipids are being intensively investigated as potential carrier systems, in particular for lipophilic substances (Nasir *et al* 2008). The microspheres system is a newly introduced lipid-based carrier system developed for parenteral and topical drug delivery of bioactive compounds. The solid phase porous

microsphere is a vehicle technology comprising inert, porous, polymeric spherical microparticles designed to entrap active ingredient, allowing for a slower rate of delivery into skin. The term microspheres describe a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles (Dahiya and Gupta, 2011; Yellanki *et al* 2010). They can also be defined as a structure made up of continuous phase of one or more miscible polymers in which the particulate drug is dispersed at the macroscopic or molecular level. Microsphere based drug delivery systems

have received considerable attention in recent years. The following advantages make them a promising means for the delivery of the nonsteroidal anti-inflammatory drugs (NSAIDs). Microspheres provide constant and prolonged therapeutic effect, which will reduce the dosing frequency and thereby improve the patient compliance. They could be injected in to the body due to the spherical shape and smaller size. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects. Microsphere morphology allows a controllable variability in degradation and drug release (Del Rosso, 2009). Topical administration of therapeutic agents offers many advantages over oral and intravenous administrations (Parsaee *et al* 2002). One of the major disadvantages in percutaneous drug delivery is its low normal drug penetration rate through the skin. Several materials have been explored to increase the penetration of drugs, including the use of enhancers, such as surfactants, solvents, azone, essential oils, terpenes and lipids. In most lipid formulations, the active substance is incorporated into waxes (Yokomiza, 1996). Wax is a fatty substance that contains long hydrocarbon chains with or without functional group (Vali *et al* 2005). Functional groups that are often present in waxes include alcohol, ester, ketone, and aldehyde. The wax esters derived from plants, insects and marine animals are used in various industrial applications (e.g. cosmetics, lubricants, polishes, surface coatings, inks, and foods) (Nishihata *et al* 1987). Rice (*Oryza sativa*) bran wax (RBX) is a natural plant wax derived from rice bran, which is a by product of rice milling. It is a major wax resource in East Asia, where rice is the main food. The potential applications of RBX in the cosmetic, pharmaceutical, food, polymer, and leather industries are as cost-efficient as those of other plant waxes, such as carnauba wax and candelilla wax (Grace *et al* 1999). Diclofenac diethylamine, a phenyl acetic acid derivative, is a potent member of the non-steroidal anti-inflammatory drugs (NSAIDs), which, due to its gastrointestinal disturbances, is topically administrated in the form of a 1.16% gel. In recent years, there have been many *in vitro* reports on lipid-based NSAID formulations, such as aqueous gel forms of Diclofenac (Gowda *et al* 2010), indomethacin gel ointment containing lipids, niosomal Diclofenac and pluronic lecithin organo-gel of Diclofenac

(Iannuccelli *et al* 2006). The later provides an affective short-term reduction in elbow pain and wrist extensor weakness associated with chronic lateral epieondylitis. The aim of present work was to formulate diclofenac diethylamine microspheres using wax to be applied topically on the skin for the purpose of sustaining its release and to avoid the side effects resulting from the oral administration of this drug and also to reduce the dosing frequency.

EXPERIMENTAL

Materials

Diclofenac diethylamine B. P. was provided as a gift sample by Aarti Drug Limited, Mumbai, India. Rice bran wax was obtained from Bajaj Mills, Warangal, Andhra Pradesh, India. Carbopol 934, methyl paraben, propyl paraben, sodium metabisulphite, sodium hydroxide, glycerin and triethanolamine was procured from Loba Chemie Laboratories, Mumbai, India. Dialysis membrane (12,000-14,000 molecular weight cut off) was procured from Hi media Laboratories, Mumbai, India. All chemicals used were of either analytical or pharmaceutical grade.

Purification of wax

The crude wax (100 g) was soxhleted with ethyl acetate (300 ml) for 30 min at 85°C. The mixture in thimble was cooled up to 25°C and was subjected to decolorization with 2% hydrogen peroxide at 90°C for 1 h and secondary decolorization with sodium chloride (15% w/v) at 100°C for 1 h (Gowda and Shivakumar, 2007). The purified wax obtained was then used for further study.

Characterization of purified wax

The rice bran wax obtained after purification was standardized to determine its physicochemical properties like solubility, melting range, acid value, peroxide value, saponification value and iodine value as per pharmacopeial procedure (IP, 2007).

Preparation of microspheres by modified cooling induced solidification method

Rice bran wax was melted in beaker on hot plate at a temperature 80-85°C. The drug was dispersed in melted wax; to this dispersion Tween 80 was added and stirred thoroughly. This dispersion was added to distilled water (80-85°C). The mixture was stirred (Remi stirrer, India) for 10 min at 1000 rpm. Then this mixture

was cooled at 1-8°C with stirring for 15 min. Then the microspheres were filtered by vacuum filtration and air dried at room temperature for 48 h (Gowda *et al* 2010).

Characterization of microspheres

Particle size

The size of the microspheres was determined by using optical microscopy (Motic microscope, B3, DM0506-04254, China).

Scanning electron microscopy

The detailed surface characteristics of the microsphere formulation were observed using a scanning electron microscope (Model JEM-100S, Japan). The microspheres sample was attached to the specimen holder using a double coated adhesive tape and gold coated (~20 nm thickness) under vacuum using a sputter coater (Model JFC-1100, Jeol, Japan) for 5–10 min at 40 mA and then investigated at 30 kV.

Rheological studies

The measurement of viscosity of the prepared gel was done with Brookfield viscometer (Brookfield RVT viscometer). The gels were rotated for 2 min at different speed for selected spindle 7.

Entrapment efficiency

The entrapped drug concentration was determined by lysis of the microspheres with phosphate buffer pH 7.4 with sonication. Accurately weighed amount of loaded microspheres (25 mg) was dissolved in 25 ml phosphate buffer pH 7.4. The solution was sonicated for 15 min and extracted for 12 h. An aliquot of 1 ml of this solution was added to 10 ml of phosphate buffer pH 7.4. The concentration of Diclofenac diethylamine in phosphate buffer pH 7.4 was determined spectrophotometrically at 276 nm. Each sample was analyzed in triplicate. The entrapment efficiency was calculated through the following relationship:

$$\text{Entrapment Efficiency} = \frac{\text{Entrapped drug}}{\text{Total Drug}} \times 100 \quad \text{Eq 1}$$

In vitro drug release study

USP dissolution apparatus, Type I was used to study the percentage of drug release from the prepared microspheres. Accurately weighed quantities of drug loaded microspheres of each batch were taken in 900 ml dissolution medium

(phosphate buffer pH 7.4) and stirred at 100 rpm by maintaining at a temperature of 37±0.5°C. The drug concentrations were determined by withdrawing the 5 ml of aliquots periodically at an interval for the next hours. Release studies were carried out in triplicate.

Experimental design

A 3² full factorial design was used for optimization of the drug loaded microspheres. The independent variables were the concentration of rice bran wax (X₁) and concentration of Tween 80 as a channelling agent (X₂) was shown in (Table 1). The minimum particle size, maximum percentage entrapment efficiency and maximum drug release were selected as dependent variables.

Table 1. Composition of optimization batches

Formulation	Coded value*	
	X ₁	X ₂
O ₁	-1	-1
O ₂	-1	0
O ₃	-1	1
O ₄	1	-1
O ₅	1	0
O ₆	1	1
O ₇	1.25	-1
O ₈	1.25	0
O ₉	1.25	1
Code value	Actual value	
	X ₁ (%)	X ₂ (%)
-1	0.75	1.0
0	1.0	1.4
1	1.25	1.8

*X₁ = conc. of wax, X₂ = conc. of channelling agent

Optimization study

Design expert software (Design expert trial version 8.0.4 StatEase Inc., USA) was used to carry out the optimized studies. A statistical second order model including interaction and polynomial terms was generated for all the response variables using multiple regression analysis (MLRA). The general form of the model is represented in equation below:

$$Y = B_0 + B_1X_1 + B_2X_2 + B_3X_1X_2 + B_4X_1^2X_2 + B_5X_1^2X_2^2 \quad \text{Eq 2}$$

where B₀ is the arithmetic average of all the quantitative outcomes of nine runs and B₁ and B₂ are the coefficients computed from the observed

experimental values of Y. X_1 and X_2 are the coded levels of independent variables. The interaction terms (X_1 and X_2) shows how the response values changes when the two factors are simultaneously changed. The analysis of variance (ANOVA) is performed to identify the insignificant factors and reduce the equation to get the better fit and the best formulation possible. The statistical validity of polynomials was established on the basis of Yate's ANOVA provision in the Design expert software. A grid search was performed to locate the composition of the formulations.

Preparation of gel containing microspheres

The weighed amount of Carbopol 934 powder was slowly dispersed in 50% distilled water with vigorous stirring, so as to avoid the clump formation and air entrapment. After complete

dispersion, the gel solution was kept in dark for 24 h for complete swelling of Carbopol. Accurately weighed amount of Diclofenac diethylamine microspheres was then incorporated in Carbopol gel with continuous stirring. In another beaker other ingredients were dissolved in remaining 25% distilled water with moderate heating and stirring. The above solution was added slowly into the Carbopol gel containing Diclofenac diethylamine with continuous stirring using magnetic stirrer. Care has been taken during formulation to avoid entrapment of air bubbles in a gel. Finally formed gel was then neutralized by sufficient quantity of sodium hydroxide solution (1% w/v). Final volume of formulation was adjusted by remaining amount of distilled water. Different concentrations of Carbopol formulation were shown in **Table 2**.

Table 2. Formulations of diclofenac diethylamine gel

Sr. No.	Ingredients	Quantities (% w/w)		
		F1	F2	F3
1.	Microsphere of Diclofenac diethyl amine B.P. (Equivalent to 1% diclofenac sodium)	1.16	1.16	1.16
2.	Carbopol 934	0.5	1	1.5
3.	Glycerine	5	5	5
4.	Triethanolamine	2	2	2
5.	Sodium metabisulphite	0.1	0.1	0.1
6.	Methyl paraben	0.18	0.18	0.18
7.	Propyl paraben	0.02	0.02	0.02
8.	Sodium hydroxide	q.s.	q.s.	q.s.
9.	Distilled water	q.s.	q.s.	q.s.

Characterization of gel

The prepared gel formulations containing drug loaded microspheres were inspected visually for their colour, homogeneity, consistency. The pH of various gel formulations was determined by using digital pH meter. Gel (1 g) was dissolved in 100 ml distilled water and stored for 2 h. The measurement of pH of each formulation was done in triplicate and average values were calculated. Spreading coefficient was determined by apparatus which consisted of a wooden block, that was attached to a pulley at one end. Spreading coefficient was measured on the basis of 'Slip' and 'Drag' characteristics of gels. A ground glass slide was fixed on the wooden block. An excess of gel (about 2 g) under study was placed on this ground slide. The gel preparation was then sandwiched between this

slide and second glass slide having same dimension as that of the fixed ground slide. The second glass slide is provided with the hook. Weight of 500 mg was placed on the top of the two slides for 5 min to expel air and to provide a uniform film of the gel between the two slides. Measured quantity of weight was placed in the pan attached to the pulley with the help of hook. Time in seconds taken by two slides to slip off from gel and placed in between the slides under the direction of certain load. Lesser the time taken for separation of two slides, better the spreadability. It is calculated by using the formula:

$$S = M \times L / T \quad \text{Eq 3}$$

where M = weight tied to upper slide,

L = length of glass slide

T = time taken to separate the slide

The viscosity of different gel formulation was determined by using a Brookfield viscometer (RVT model) with spindle number 7.

Drug content

Drug content in gel was measured by dissolving known quantity of gel (1 g) in suitable quantity of phosphate buffer pH 7.4. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured at 276 nm using spectrophotometer. Drug content was calculated by using the calibration curve.

In vitro drug release studies

The *in vitro* drug release studies were carried out using modified Franz diffusion cell (effective diffusion area 2.54 cm² and 20 ml cell volume). The formulation was applied on dialysis membrane which was placed between donor and receptor compartment of the Franz diffusion cell. Phosphate buffer pH 7.4 was used as a dissolution media. The dialysis membrane was presoaked in phosphate buffer pH 7.4 for 24 h. The temperature of the cell was maintained at 37°C by circulating water jacket. This whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. The samples (1 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV visible spectrophotometer (JASCO, V-630, Japan) at

276 nm after appropriate dilutions.

Stability studies

The prepared gels were packed in aluminium collapsible tubes and subjected to stability studies at 40 ±2°C/75±5% RH as per ICH guidelines for a period of 3 months. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance, pH, rheological properties, drug content and *in vitro* drug release.

RESULTS AND DISCUSSION

Recently waxes are reported for preparation of microspheres to release the entrapped drug in the stratum corneum. In the present study, a novel meltable dispersion emulsified cooling induced solidification method was employed using rice bran wax (FDA approved) material and non-toxic solvents to entrap the drug. The rice bran wax procured from the mill was not purified therefore purification of the wax was carried out as per reported method. The purified wax characterized for solubility, melting range, acid value, peroxide value, saponification value and iodine value. **Table 3** summarizes characteristics of purified wax. The wax was insoluble in acetone, ethanol, soluble in chloroform. The acid value was low indicating lesser degree of unsaturation, lower iodine value showed less degree of unsaturation. High saponification value showed short fatty chain and low saponification value indicated long fatty chain in wax. Higher peroxide value showed higher free radicals which damage the tissues.

Table 3. Properties of rice bran wax

Test	Unorganized wax	Organized wax
Solubility	Practically insoluble in acetone, ether, ethanol but freely soluble in chloroform	Practically insoluble in acetone, ether, ethanol but freely soluble in chloroform
Melting range	82°-85°C	78°-82°C
Acid value (ml/g)	25.94	9.44
Peroxide value (mEq/kg)	63.8	24.43
Saponification value	168.3	77.13
Iodine value	7.0	7.0

Preparation of microspheres

However in the present study, various parameters were optimized such as wax ratio, stirring speed, stirring time, amount of tween 80 and rapid cooling during the preparation of wax microspheres. Where the ammonia buffer having

the pH 9 used as external phase, the solubility of the drug was reduced and the encapsulated amount of the drug in microspheres was increased. But when these microspheres were dispersed in Carbopol gel the release of ammonia traces were observed in gel. The

maximum drug load was obtained at pH 7 by using the distilled water as an external phase. When pH value changes from 7 to 10, the percent of drug loading was reduced for drug loaded wax microspheres.

Incorporation of drug into wax microspheres and drug release from microspheres requires the addition of a Tween 80 at an optimum concentration to reduce the interfacial tension between the hydrophobic material and external aqueous phase. An attempt was made to incorporate drug in the wax microspheres without the addition of a surfactant. But the process was failed and it resulted in an aggregate cake like mass during the solidification of wax. It may be due to repulsion resulting from high interfacial tension between the hydrophobic waxy material and external aqueous phase. It was found that surfactant having a HLB value of 15 or more was suitable to increase substantially dispersion of wax and promote drug incorporation in the microspheres. To obtain an optimal surfactant concentration, various concentrations ranging from 1 to 2% w/w of the total formulation were tested. At low concentration of Tween 80 resultant wax microspheres were composed of irregular masses, which were not possible to distinguish as individual microspheres. Optimal concentration of Tween 80 (1.8% w/w) was used to produce solid, discrete microspheres with good flow properties. A similar surfactant concentration was reported for beeswax microspheres prepared by a meltable dispersion method (Gowda *et al* 2010).

In the present study, to produce the spherical discrete microspheres, an optimum drug to wax phase ratio of 1:1 w/w was used. It was found that higher the amount of drug to wax ratio (1:2) produces aggregate masses during the cooling process. It may be due to more amounts of excess wax leads to aggregate masses. Microscopic study also indicated the presence of the irregular shape of microspheres and resulted microspheres were unsuitable for pharmaceutical use. The important factor that influences the size distribution of microspheres was stirring speed and stirring time. An optimum stirring speed of 1000 rpm and time was 10 min was used to obtain reproducible microspheres. It was observed that with the increase in the stirring speed from 1000 to 1300 rpm, there was a decrease in the average size and recovery yield of the microspheres, small

sized wax microspheres which were lost during successive washing and filtration. When the stirring speed was lower than 1000 rpm, larger aggregates were formed. An increased stirring time from 10 to 15 min (at a stirring speed of 1000 rpm), decreased recovery yield of microspheres was observed. Because more stirring speed for longer duration lead to produces smaller sized microspheres and these microspheres were lost during successive washing and filtration. When the stirring time was less than 10 min, some amount of melted material was adhered to the sides of the beaker during the cooling process, resulted in lower recovery of yield.

Reproducibility batches at optimum parameters shows reproducible results. In the present study, 150 ml (optimum amount) of aqueous phase was used to produce spherical microspheres. Resultant microspheres free from surface irregularities and free flowing in nature. When the volume of external phase was increased (>150 ml), then the resultant microspheres were having surface irregularities (confirmed by microscopic studies), sticky, aggregate and impossible to distinguish as an individual microspheres. A rapid cooling study during the preparation of wax microspheres was carried out. The temperature of the mixture was cooled rapidly (<15 min) and brought down to 10°C by the cold water, produced microspheres were solid, discrete, free flowing in nature. When, the temperature of the mixture was cooled slowly (>15 min), microspheres obtained were flaky, sticky, aggregate in nature, not suitable for pharmaceutical purpose. Temperature of the aqueous phase was maintained 5°C higher than the melting point of the wax in the corresponding formulations. From microscopic studies it was observed that the resultant microspheres were free from surface irregularities (**Figure 1**) except some wrinkles. But, when the temperature of the aqueous phase was less than the 5°C than the melting point of the wax, big flakes were produced. A total of five formulations were prepared by varying amount of drug to polymer ratio from 0.25 to 1.25% w/w. From evaluation, it was observed that drug loading, entrapment efficiency and particle size increased with increasing concentration of wax. From observation it was found that the drug to wax ratio and concentration of tween 80 affected entrapment efficiency, particle size and drug release when stirring speed, stirring time, rate of

cooling and volume of aqueous phase were kept optimum. So, these two factors (drug to wax

ratio and concentration of tween 80) were further studied using factorial design.

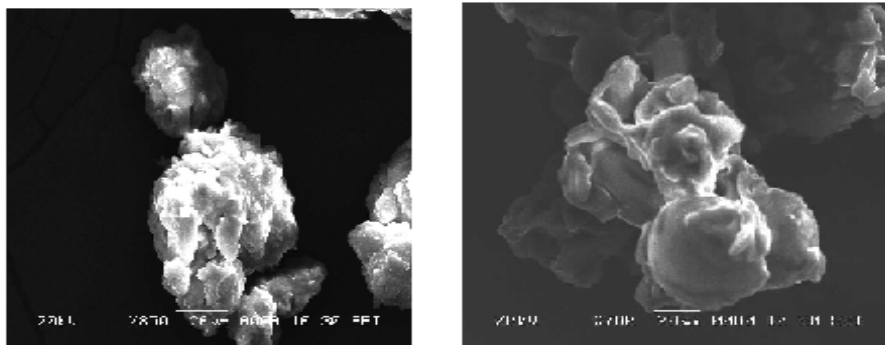


Fig. 1. SEM photograph of microspheres of rice bran wax

Optimization of formulation

In this study, concentration of wax (X_1) and concentration of Tween 80 (X_2) were selected as independent variables whereas particle size (Y_1), drug release (Y_2) and entrapment efficiency (Y_3) was selected as dependent variables. A 3^2 randomised full factorial design was used in this study. In this design two factors were evaluated, each at 3 levels. Concentration of wax % w/w was taken at 0.75 (Low), 1.00 (Medium) and 1.25 (High) at 3 levels. Concentration of tween 80% w/w was taken at 1.00 (Low), 1.40 (Medium) and 1.80 (High) at 3 levels. The nine optimization batches (O_1 - O_9) were prepared in 3^2 full factorial designs and evaluated.

Diclofenac diethylamine microspheres (batch O_1 - O_9) were evaluated for drug release study. After analysis of both independent variables and dependent variables by Design expert software, the results showed that 0.92% w/w concentration of wax and 1.17% w/w concentration of tween 80 were optimum for producing microspheres particle size less than 50 μ m, maximum entrapment efficiency and sustained release for 12 h. Batches with these concentrations were prepared and evaluated. Particle size was 38.02 μ m, entrapment efficiency 59.09% and drug release 92.81% which were significant. Drug release kinetic showed that release followed Higuchi model, this suggested microspheres as matrix system.

Evaluation of gel containing microspheres

Physical examination

The gel containing microspheres formulations were clear and transparent with a smooth and homogeneous appearance. The pH values of all prepared formulation ranged from 6.2 to 6.5, which were considered acceptable to avoid the

risk of irritation upon application to the skin because adult skin pH is 5.5 to 6.5. The spreadability of various gel formulations was also good.

Rheological studies

The measurement of viscosity of the prepared gel was done with Brookfield viscometer (Brookfield RVT viscometer). The gels were rotated for 2 min at different speed for selected spindle 7. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained in the range of 5000 cps to 20000 cps.

Drug content

Drug content was calculated using the equation, which was obtained by linear regression analysis of calibration curve. The drug content was observed in the range of 95.62 to 98.98%.

In vitro drug release

In vitro drug release study is shown in **Figure 2**. At low concentration of wax, drug release was 92.059 % in 6 h (for A_1 batch, drug: wax, 1: 0.25), as wax concentration was increased the drug release was extended with concentration of wax. It was observed that at 1:1 concentration of Drug: wax (A_4) the drug release was 86.205 % at 12 h; further increasing concentration of wax drug release was extended.

The *in vitro* release kinetics for all the batches are shown in **Table 4**. Korsmeyer-Peppas equation was followed for *in vitro* drug diffusion from gel containing microspheres. From diffusion coefficient of Korsmeyer-Peppas equation indicated that the non-Fickian mechanism was basically involved in the drug release from gel containing microspheres.

Stability studies

Accelerated stability studies were analyzed for *in vitro* diffusion profiles (Table 5). From stability

studies of optimized batches, it was found that gel remained stable even after exposing to high temperature/moisture conditions.

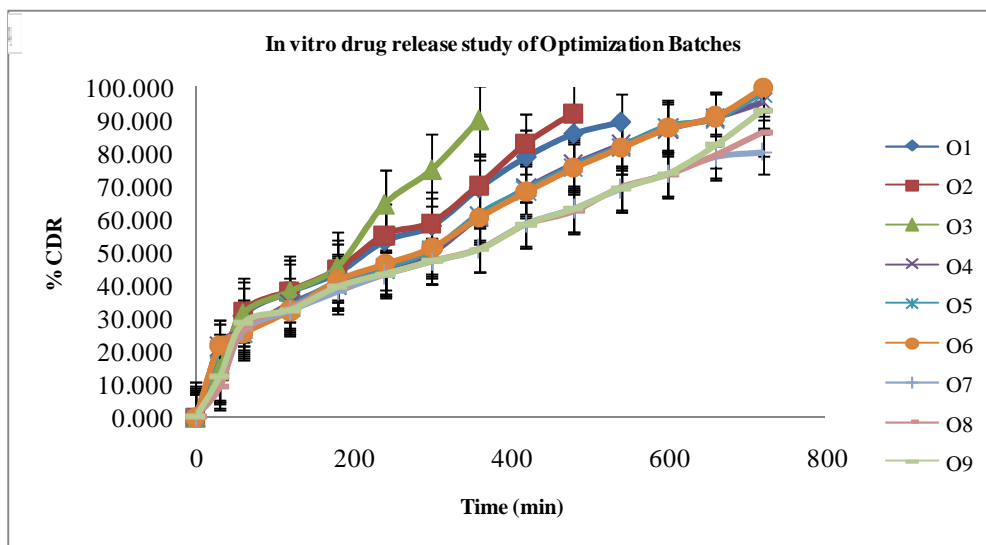


Fig. 2. *In vitro* drug release study of optimization batches

Table 4. *In vitro* drug release kinetics study

Batch code	Zero Order		First Order		Higuchi		Hixon Crowell		Korsemeyer Peppas	
	r ²	K ₀	r ²	K ₁	r ²	K ₃	r ²	K ₄	r ²	K ₅
O1	0.938	0.187	0.979	-0.0037	0.988	3.668	0.988	-0.001	0.981	2.007
O2	0.946	0.204	0.945	-0.0042	0.981	3.7777	0.974	-0.0011	0.978	2.041
O3	0.971	0.260	0.949	-0.005	0.971	4.153	0.977	-0.0013	0.981	1.614
O4	0.935	0.151	0.977	-0.003	0.986	3.403	0.983	-0.0008	0.935	3.431
O5	0.933	0.151	0.964	-0.003	0.989	3.412	0.988	-0.0009	0.986	3.131
O6	0.921	0.151	0.914	-0.004	0.994	3.413	0.980	-0.0009	0.993	3.245
O7	0.913	0.128	0.988	-0.002	0.992	2.918	0.979	-0.0006	0.973	1.680
O8	0.919	0.130	0.974	-0.002	0.988	2.956	0.978	-0.0006	0.967	1.639
O9	0.925	0.134	0.922	-0.002	0.982	3.026	0.976	-0.0007	0.959	2.330

Table 5. Results for stability studies

Sr. No.	Evaluation Parameter	Initial	First month		Third month	
			40°C/75% RH	30°C/60% RH	40°C/75% RH	30°C/60% RH
1.	Appearance	Clear, transparent	Clear, transparent	Clear, transparent	Clear, transparent	Clear
2.	Homogeneity	Smooth	Smooth	Smooth	Smooth	Smooth
3.	pH	6.3	6.1	6.3	6.4	6.3
4.	Spreadability (g.cm/sec)	15.0	16.07	16.07	14.06	14.06
5.	Viscosity (cps)	Minimum speed	8000	8000	8000	8000
		Maximum speed	2000	1800	1800	2000
6.	Drug content (%)	98.70	99.09	98.70	99.03	98.70
7.	<i>In vitro</i> diffusion study (%)	97.23	96.92	97.10	96.03	96.89

Conclusion

It can be concluded that from the present study, the microspheres prepared from rice bran wax have good sustained release property, with increasing concentration of wax drug release was sustained. Tween 80 was required for optimum drug release profile. *In vitro* drug release kinetics clearly indicated that microspheres follows Higuchi model. Carbopol 934 was used as gelling agent for microspheres. They exhibited good homogeneity, spreadability, viscosity, diffusion characteristics. Revealing from *in vitro* drug diffusion kinetics shows

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